R E P O R T

of a

Conference to Develop a Coordinated Research Program on

Anaplasmosis of Cattle

Called by

B. T. Simms
Chief of the Bureau of Animal Industry
U. S. Department of Agriculture

February 10-11, 1948

Room 124 E - U. S. Department of Agriculture

Washington, D.C.

(Reproduced - May, 1962)

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UNITED STATES DEPARTMENT OF AGRICULTURE
Agricultural Research Administration
Bureau of Animal Industry
Washington 25, D. C.

ZM - 1.229

January 19, 1948

TO DIRECTORS OF EXPERIMENT STATIONS, STATE LIVESTOCK SANITARY OFFICIALS, RESEARCH WORKERS, AND OTHERS:

At the conference of Research Workers in Animal Diseases in the Southern States held at the Regional Animal Disease Research Laboratory, Auburn, Alabama, March 20-21, 1947, anaplasmosis was one of the principal subjects of discussion. Dr. Hawkins of the Oklahoma Experiment Station discussed the Flannagan-Hope Bill with particular reference to regional projects. A committee was appointed to develop a regional plan for research on anaplasmosis.

The Bureau has been considering for some time the calling together of research workers interested in anaplasmosis to develop a national coordinated program. Following the meeting at Auburn, discussions were had with Dr. Hawkins and others relative to the calling of such a meeting to consider a national research program on anaplasmosis. This was considered to be quite advisable and it was the opinion of some that the meeting should be held in connection with the Southern Agricultural Workers in February 1948. The Bureau is, therefore, calling a meeting in Washington, D. C., February 10 and 11, 1948, to develop such a program. The meeting will be held in Room 124-E Administration Building, U. S. Department of Agriculture, starting at 10:00 a.m.

There is attached a program of the meeting listing the subjects for discussion. Representatives from each State should come to the meeting prepared to discuss any or all of the subjects on the agenda and to have prepared at least a tentative program of what the States might do on anaplasmosis research, consistent with present facilities and funds, or with the facilities and funds that might be required. The hope is to develop a long-range program which can be used as a basis for obtaining the necessary funds in order to develop the information necessary to control this costly cattle disease.

Invitations to this meeting are being extended to the State Experiment Stations and State Livestock Sanitary Officials of States where the disease is considered to be of importance or potential importance.

Very truly yours,

B. T. Simms
Chief of Bureau

Enclosure

ZM-1.229 Sent to:

ALABAMA
Dr. M. J. Funchess
Dr. R. S. Sugg
Dr. I. S. McAdory

ARKANSAS
Dr. L. S. Ellis
Dr. J. S. Campbell
Dr. Delbert Swartz

CALIFORNIA
Dr. C. B. Hutchison
Dr. A. K. Carr
Dr. C. M. Haring

COLORADO
Mr. H. J. Henney
Dr. R. M. Gow
Dr. I. E. Newsom

DELAWARE
Dr. G. L. Schuster
Dr. Harry McDaniel

FLORIDA
Dr. Harold Mowry
Dr. J. V. Knapp
Dr. A. L. Shealy

GEORGIA
Dr. H. P. Stuckey
Dr. T. B. Clower
Dr. Thomas J. Jones

IDAHO
Dr. R. E. Theophilus
Dr. Arthur P. Schneider
Dr. G. C. Holm

KANSAS
Dr. R. I. Throckmorton
Dr. R. R. Dykstra
Dr. Will J. Miller

LOUISIANA
Dr. E. P. Flower
Mr. W. G. Taggart
Dr. W. T. Oglesby

MARYLAND
Dr. W. B. Kemp
Dr. A. L. Brueckner
Dr. Hendershott

KENTUCKY
Dr. F. E. Hull
Dr. B. F. Pigg

OKLAHOMA
Dr. D. H. Ricks
Mr. W. L. Blizzard

WYOMING
Dr. A. M. Lee

NEW MEXICO
Mr. A. S. Curry
Mr. Sam McCue
Dr. J. W. Brenner

ARIZONA
Dr. Frank D. McMahon
Dr. P. S. Burgess
Dr. W. J. Pistor

MISSISSIPPI
Dr. H. C. Simmons
Dr. Russell Coleman
Dr. J. W. Scales

MISSOURI
Mr. E. A. Trowbridge
Dr. H. E. Curry
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Dr. J. H. Hilton
Dr. L. J. Faulhaber
Dr. C. D. Grinnels

SOUTH CAROLINA
Dr. H. P. Cooper
Dr. R. A. Mays
Dr. R. O. Feeley

TEXAS
Dr. R. D. Lewis
Dr. Jim L. Adrian
Dr. H. Schmidt

VIRGINIA
Dr. H. N. Young
Dr. H. C. Givens
Dr. I. D. Wilson

WYOMING
Dr. J. A. Hill
Dr. George H. Good

TENNESSEE
Dr. C. E. Brehm
Dr. C. E. Kord

List of Men in Attendance
Anaplasmosis Conference
February 10-11, 1948
Washington, D. C.

Name	Address
T. H. Applewhite	USDA, BAI, Jacksonville, Fla.
F. C. Bishopp	USDA, BEPQ, Washington, D. C.
W. H. Boynton	Univ. of California, Berkeley, Calif.
A. L. Brueckner	Univ. of Maryland, College Park, Md.
C. L. Davis	USDA, BAI, Denver, Colo.
G. Dikmans	USDA, BAI, Beltsville, Md.
Ervin A. Eichhorn	USDA, BAI, Washington, D. C.
Herman Farley	Okla. A&M College, Stillwater, Okla.
D. W. Gates	USDA, BAI, Beltsville, Md.
L. T. Giltner	USDA, BAI, Washington, D. C.
Charles G. Grey	USDA, OES, Washington, D. C.
A. H. Groth	USDA, BAI, Auburn, Ala.
Louis E. Hawkins	Oklahoma A&M College, Stillwater, Okla.
D. E. Howell	Oklahoma A&M College, Stillwater, Okla.
Howard W. Johnson	USDA, BAI, Beltsville, Md.
J. V. Knapp	State Vet., Tallahassee, Fla.
Aubrey M. Lee	USDA, BAI, Washington, D. C.
J. C. Lotze	USDA, BAI, Beltsville, Md.
W. M. MacKellar	USDA, BAI, Washington, D. C.
H. W. Marston	USDA, Washington, D. C.
Z. A. Massey	OES, Experiment, Ga.
Wm. M. Mohler	USDA, BAI, Washington, D. C.
Lawrence O. Mott	USDA, BAI, Beltsville, Md.
Paul L. Piercy	La. State Univ., Baton Rouge, La.
L. J. Poelma	Univ. of Maryland, College Park, Md.
E. W. Price	USDA, BAI, Beltsville, Md.
Lee M. Roderick	Kansas State College, Manhattan, Kan.
Dorsey A. Sanders	OES, Gainesville, Fla.
H. Schmidt	A&M College, College Station, Tex.
H. W. Schoening	USDA, BAI, Washington, D. C.
B. T. Simms	USDA, BAI, Washington, D. C.
H. H. Stage	USDA, BEPQ, Washington, D. C.
Clarence A. Woodhouse	Univ. of Delaware, Newark, Del.

Conference to Develop a Coordinated Research Program on Anaplasmosis
February 10-11, 1948

Bureau of Animal Industry, ARA
U. S. Department of Agriculture
Washington, D. C.

1. Purpose of meeting
2. The present extent of anaplasmosis in the United States
3. Subjects for discussion for formulation of a national research program on anaplasmosis

   a. Distribution, extent, and losses caused by the disease
      1. How can this information best be obtained

   b. The causative agent
      1. Physical and chemical properties
      2. Filtrability - artificial culture
      3. Modes of transmission
      4. Virulence and its modifications
      5. Susceptibility of species
      6. Other aspects

   c. Diagnosis of the disease
      1. Clinical
      2. Pathological
      3. Animal inoculations
      4. Serological
      5. Differential diagnosis
      6. Other methods

   d. Therapeutics
      1. Chemotherapy
      2. Other methods

   e. Prevention
      1. Immunization and premunition
      2. Control of vectors
      3. Elimination of mechanical transmission

   f. Control of the disease
      1. Study of methods of control under field conditions

   g. Formulation of program

Conference to Develop a Coordinated Research Program on Anaplasmosis

Attached are a copy of letter ZM-1.229 calling a meeting of Directors of Experiment Stations, State Livestock Sanitary Officials, research workers and others to develop a national coordinated program on anaplasmosis research, and a copy of the agenda for such program. There is also attached a list of those who received the letter and agenda. The meeting was held in Washington, D. C., February 10-11, 1948.

Dr. Schoening discussed the purpose of the meeting and called on members present to give available information on the extent of the disease in their respective States:

Dr. Farley of Oklahoma stated that it was difficult to determine the extent of anaplasmosis but that it was prevalent in the northeast, east, central, and south but not in the southwest. More prevalent in 1947 than ever before.

Dr. Roderick of Kansas: Prevalent in southeast and south.

Dr. Brueckner of Maryland: On Eastern Shore and to some extent elsewhere.

Dr. Knapp of Florida: In all counties, but unrecognized in some.

Dr. Schmidt of Texas: Much infection.

Dr. Boynton of California: Southern counties, but reports not complete.

Dr. Piercy of Louisiana: No accurate means of reporting; mostly in the south where insects are reported from all parishes.

Dr. C. L. Davis, Colorado: Prevalent but believes authorities should make disease reportable; packing house veterinarians and practitioners send in slides and furnish much information.

Dr. Lee, Wyoming: Diagnosed in 10 counties; suggests sending out questionnaire such as is used in equine encephalomyelitis.

Dr. Sipos, Virginia: Present in some southern and eastern counties.

Dr. Schoening: Suggests States should make maps and charts indicating where disease exists. In 1947 the disease was present in 28 States--Virginia, North Carolina, South Carolina, Florida, Georgia, Alabama, Mississippi, Tennessee, Louisiana, Arkansas, Texas, Oklahoma, Kansas, Missouri, Iowa, Colorado, Wyoming, Montana, New Mexico, Arizona, Illinois, Nevada, Idaho, Oregon, California, Maryland, Delaware.

Dr. Piercy, Louisiana: Uses splenectomized calves for diagnosis of carrier, interested in biochemistry of the disease, treatment, sterilizing carriers.

Dr. Sanders, Florida: Treatment, control and prevention; insecticide spraying.

Dr. Roderick, Kansas: Medicinal treatment, diagnosis.

Dr. Brueckner, Maryland: Sterilization of carriers, wants to try complement-fixation test; is working with Bureau of Animal Industry on survey.

Dr. Schmidt, Texas: Saving the animals by a cure; diagnosis of carriers, premunition.

Dr. Sipos, Virginia: Locating the disease, helping local veterinarians.

Dr. Farley, Oklahoma: Diagnosis, treatment and prevention.

Dr. Mott, BAI: Study the infective agent, serological diagnosis.

Dr. Dikmans, Zoological Division, BAI: Test drugs experimentally at the beginning of the disease, review of drugs already used.

Lotze, Mott, and others: Blood chemistry increase in globulin.

Dr. Howell: Vectors (control) DDT, BHC, Chlordane, about 1% kill on house flies, DDT and chlordane do not kill replete tick but BHC does. Parathion too toxic, do not have anything against horse flies.

February 5, 1948

Anaplasmosis Vaccine Immunity Studies

by

Lawrence O. Mott and Daniel W. Gates
Pathological Division, Bureau of Animal Industry

Most investigators working with either human or animal protozoan diseases have encountered considerable difficulty in demonstrating the production of immunity from vaccination against these diseases without infecting the vaccinated individual. Anaplasmosis from this standpoint appears to fit perfectly into this "sometimes disputed" protozoan classification group. Many vaccines have previously been prepared against anaplasmosis by Bureau and other workers, with little evidence of immunity response except where live vaccine (premunition tests) were used and all animals were infected and became lifelong carriers of the disease.

Since March 1944 the authors have artificially infected over 60 cattle with anaplasmosis for antigen production alone. Most of these animals were exposed in passage series; the course of the disease in each animal was carefully checked for temperature, red cell volume, parasite count, clinical reactions; and serum samples were saved for complement-fixation studies. Some of these animals died, and 3 cases recovered to become carriers; however, the remaining infected cases were killed near the peak of the parasite invasion of the red cells, and their blood was then harvested for antigen production. There were 26 antigens prepared, a part of each lot of which was injected into one or more cattle as each antigen was completed. None of these animals became infected from the antigen injections, and most all were later exposed to anaplasmosis along with unvaccinated controls to test these animals for immunity in vaccine tests 1 and 2.

Vaccine Test Number 1

There were 6 non-splenectomized cows, 7 to 8 years old, that had been vaccinated with from 1 to 5 doses of antigen totaling from 200 cc to 2,775 cc for each animal which were exposed 1 to 3 months later along with one 5-year-old, non-splenectomized, control cow. A massive exposure dose of 400 cc was given intravenously from an acute case having 20 percent of the red cells parasitized and a red cell volume of 9.5 percent. This exposure is about one million times greater than the exposure used in vaccine test number 2.

The heavy exposure was used so it could be determined in a short time if there was any evidence of immunity.

The 6 vaccinated cows were checked for pre-exposure infectivity by subinoculating 200 cc of citrate blood from each individual animal into a normal splenectomized calf. The calf was carefully checked for over 60 days and remained normal.

After exposure the control cow showed marginal bodies in 3 days, and the vaccinated cows showed marginal bodies in 9,9,11,11,12, and 14 days.

The parasite count reached 21 percent in the control cow and 3.5, 8.0, 12.0, 20.0, 21.0, and 22.0 percent in the vaccinated cows. The three vaccinated cows with the low parasite counts showed no rise in temperature nor clinical evidence of being sick. The control cow showed the most marked temperature reactions. Three of the vaccinated cows showed a temperature reaction, but only one cow became visibly sick and went off feed.

The red cell volume of the control cow dropped the lowest, 10 percent, during which time she was down for a couple of days, and it was thought she was going to die. The red cell volume of the vaccinated cows all showed a marked drop to a minimum low of 24.0, 19.0, and 14.5 percent in the 3 non-clinical vaccinated cows and to 16.0, 14.5, and 10.5 percent in the three vaccinated cows that showed a temperature and had the highest parasite count-- the 10.5 percent being shown by the one vaccinated cow that was sick.

The results of this test show evidence of immunity being conferred by all the vaccines and a marked immunity by some of the vaccines as all of the more susceptible 7- and 8-year-old vaccinated cows had longer incubation periods, higher red cell volumes, and less temperature reaction than the 5-year-old control cow.

Vaccine Test Number 2

There were 12 vaccinated cows and 3 control cows used in vaccine test number 2, ten of the vaccinated animals were vaccinated at about the same time with dosages similar to those in test number 1, and 3 of these vaccinated animals received the same vaccine as was injected into some of the vaccinated animals in test number 1. The main differences in this test from the preceding one were (1) a lowering of the exposure dosage to somewhere near what was thought to be a natural exposure dose and (2) making the exposure 3 months later than the date of the first exposure.

The exposure of these animals was delayed so long that we were skeptical of any immunity remaining, so the 10 vaccinated animals were divided into two groups of 5 animals each. One group was re-vaccinated, and the other group was not retreated. At this time 2 new cows were added to the experiment, each of which received one of the new spleen vaccines which the re-vaccinated group of cows received.

The results of vaccination test number 2 showed that:

1. Four vaccinated cows (or 33.3 percent) resisted infection. This was proved by subinoculation of their blood into splenectomized calves that remained normal.

2. Three vaccinated cows (or 25.0 percent) had fair immunity, having shown no clinical evidence of infection but were proved positive cases by subinoculation tests.

3. Five vaccinated cows (or 41.6 percent) had poor or no immunity.

4. Three control cows became infected, showed clinical evidence of infection and no evidence of immunity.

Any conclusions drawn from these experiments should be considered preliminary and subject to confirmation by further tests.

THE PRODUCTION OF AN ANTIGEN FOR ANAPLASMOSIS COMPLEMENT-FIXATION TESTS

By L. O. Mott and D. W. Gates
Pathological Division
Bureau of Animal Industry

The Bureau of Animal Industry has recognized the need for a good anaplasmosis diagnostic test ever since the disease was first recognized in this country. The nature of the disease is such that the acute stage of infection is followed either by anemia and death or recovery with an active immunity accompanied by the persistence of the infective agent in the recovered cases, making most all cases, carriers of the disease for life. These carrier animals appear normal and cannot be differentiated from non-infected animals in a herd so they may remain in a herd as a constant source of unknown infection.

DIAGNOSIS

The development of a practical method of diagnosis to pick out these animals would offer a means of control and eradication of the disease and has been a constant challenge to Bureau workers and other investigators.

The disease may be accurately diagnosed in acute cases by examining stained blood smears showing marginal bodies in the red blood cells when the percentage of red cells affected is 0.5 percent or more. If the percent of what appears to be marginal bodies is found in less than 0.5 percent of the red cells, a positive diagnosis is usually questionable because on numerous occasions these investigators have found a few similar bodies in tested, known susceptible, normal animals.

Anaplasmosis carrier cases may be diagnosed accurately by removing the spleen from the suspected carrier, which induces an acute flare-up of the disease accompanied by the appearance of marginal bodies in the red blood cells, or blood may be collected from the suspected carrier and injected into susceptible mature cows or calves that have had their spleens removed to make them more susceptible, following which injection these animals will develop an acute form of the disease within 60 days if the donor is infected with anaplasmosis. These diagnostic methods because of their accuracy are of inestimable value to the research worker; however, they are economically impractical for field diagnostic work. A number of other diagnostic tests have been applied to anaplasmosis with very little success. The complement-fixation test when applied to anaplasmosis on two occasions by Bureau workers gave some evidence that this test may have some practical value for diagnosis.[1,2] In both instances the antigens were reported to have shown definite antigenic value, but could not be produced in sufficient quantities for extensive studies or for practical use.

ANTIGEN PRODUCTION

The authors, recognizing the importance of this early work, started searching for practical methods of antigen production, whereby larger quantities of a satisfactory antigen for complement-fixation testing could be produced. A number of bovine crude blood antigens in considerable volume were made from

lysed, washed, citrated, red blood cells from acute anaplasmosis cases prepared according to the technique used by the Army for the production of an antigen used with the complement-fixation test for the diagnosis of malaria.[3] There were a number of difficulties encountered and overcome in the production of this antigen. The most arduous task was in connection with raising the parasite count of the red cells, the successful results of which have been of considerable value in the production of our later antigens. These bovine crude blood antigens appeared to be specific for anaplasmosis and gave some good positive and negative reactions with known positive and negative sera samples; however, the tests were somewhat difficult to read because of the high hemoglobin color content and studies were continued to eliminate this objectionable feature.

Another anaplasmosis antigen "carbon dioxide blood precipitate antigen" was then prepared according to the methods described by Heidelberger and Mayer.[4] Antigens prepared by this method from normal, and anaplasmosis-infected cows and normal horses were found to be unsatisfactory for anaplasmosis complement-fixation work. The investigators observed that a considerable amount of the antigen material was being lost during the processing with this described technique and also noted a marked difference in the results from processing of bovine and equine red cells. It was believed that possibly this same variation would be found with human cells and accounted for our loss of antigenic substance. Various changes in the technique were made and tested out to correct the faults found with the described production methods, following which time most of the antigens prepared from acute anaplasmosis cases with high parasitized red cell counts, produced a better antigen than the bovine crude blood antigen prepared from the same animal and was also free from the objectionable high hemoglobin color content.

SOURCE OF MATERIAL

The source of anaplasmosis infective material may be blood from an acute case or from a known infected carrier animal, also if carrier animals are not maintained as a source of infective material, it has been demonstrated that frozen citrated blood from an acute case stored for 13 months at-50degreesC. to-70 degrees C. resulted in an acute case with a 24-day-incubation period. A similar amount of infective material from the same acute case, drawn at the same time but injected immediately into a susceptible animal resulted in immediate parasite multiplication without an incubation period and an acute case. This demonstrates the great loss of the anaplasmosis infective agent by freezing and thawing. The inactivation of the anaplasmosis infective agent by freezing and thawing has been further demonstrated in over 90 bovine animals that were injected with processed washed infected cells from 26 acute cases that had been rapidly frozen and thawed six times before being injected. None of the 90 injected animals became infected with anaplasmosis. The use of dry-ice-stored blood from acute cases appears to have no advantage over the use of carrier blood in cutting down the beginning of production time because the incubation periods obtained with each method are practically the same.

ANTIGEN PASSAGE ANIMALS

It is believed that the best type of animals for antigen production are normal thrifty calves 12 to 18 months of age weighing from 400 to 600 pounds, either male or female, from which the spleens have been removed 30 to 60 days before the inoculation date. Younger calves are suitable but do not yield enough blood. Older animals with and without spleens have been used with some success but they have been found much less uniform in susceptibility than the younger animals. It is also advisable to use animals that have never been inoculated with any biologic product, including normal blood or serum from other normal animals, as these inoculations appear to influence the susceptibility of the calves and probably explains some of the variation in susceptibility of older animals, which usually have had some injections, vaccinations, or inoculations during their lifetime before we use them. This cause of susceptibility variation is mentioned as an important addition to the five generally attributed causes of variation reported by Lotze;[5] namely (1) age and condition, (2) individual resistance, (3) amount and character of inoculation, (4) ability of individual to regenerate red blood cells, and (5) virulence of specific agent.

INOCULATION METHODS

The inoculation of the anaplasmosis infective agent is probably one of the most important steps in the production of a good antigen. Starting with carrier blood and using a dose of 10 c.c. to 50 c.c. for each successive passage, harvested from each acute case at the peak of an acute infection, usually took four or more passages over a period of 3 or 4 months to produce a satisfactory antigen. Additional infectivity studies, covering a 2-year period in which 60 animals were used in making rapid serial passages in from 3 to 15 animals for one series, showed that one or two passages from an acute case may result in a high percent of infected red cells and produce a satisfactory antigen when massive infective doses are given intravenously. The acute donor's blood is harvested in an equal volume of Alcever's solution at or soon after the time of maximum red cell infectivity. At this time the red cell volume will usually average around 10 percent of the total blood volume. The Alcever's solution is used in place of sodium citrate anticogulant because it appears to lower the amount of hemolysis in the heavily parasitized red cells that are much more fragile than red cells from a normal animal. The suspension of red cells in Alcever's solution is then centrifuged in 250 c.c. Pyrex centrifuge bottles at 1,900 r.p.m. for 20 minutes, after which the supernatant fluid is siphoned off and the red cells given one washing by resuspending in physiological saline and centrifuging again for 30 minutes at 1,900 r.p.m. The saline serum supernatant is then drawn off, the packed red cell volume is measured and resuspended in an equal volume of physiological saline, and this parasitized red cell saline suspension is injected intravenously into the next passage animal. The size of the infecting dose should vary according to the size of the passage animal to be exposed. A dose of 500 c.c. of packed red cells suspended in 500 c.c. of physiological saline was the usual dosage selected for our average passage animal. This represents the cells from about five liters of blood from the acute donor case.

Considerable caution should always be used whenever large infective doses from acute cases are inoculated intravenously into animals being exposed, as symptoms of severe shock may start within a few minutes to a few hours following exposure and result in death of the exposed animals with the loss of the passage material. The toxic or shock-producing agent was found to be present in both the infected animal's serum and its washed red cells, so the investigators increased the margin of safety of large doses by removing the serum and resuspending the infected red cells in physiological saline before inoculating. The margin of safety was frequently further increased by giving the infective material in several doses at 1-or 2-hour-intervals. Most cases showing these symptoms of shock will respond temporarily to treatment with one intramuscular injection of epinephrine (epinephrine hydrochloride) but may die later (overnight) unless the exposed animals are kept under constant observation for a 24-hour, post-exposure period during which time epinephrine should be administered whenever symptoms of shock reappear. It is usually not necessary to administer epinephrine more frequently than once every hour in severe cases. The single dosages of 1 to 1,000 epinephrine used, ranged from 1 c.c. to 5 c.c. depending on the animal's reaction.

Satisfactory antigens have been prepared from splenectomized calves and cows and from whole cows (with spleens). It is necessary to observe the course of the disease very closely following inoculation by daily checking the thermal reaction, red cell volume, red cell parasity count, and clinical reactions, since the animal may pass the peak of the red cell parasity invasion and die before the parasitized red cells can be harvested. The observed rate of increase of the parasitized red cells in these artificially infected acute cases is approximately doubled every 24 hours and usually reaches the maximum in 7 to 9 days. The blood is usually harvested from the infected animals soon after the maximum degree of red cell parasite invasion is reached by fixing the animal in a standing position in stocks with a stanchion front. The operative area is clipped, shaved, disinfected, and locally anesthetized. The blood is collected from the carotid artery into closed flasks containing Alcever's solution, if for reinoculation, and into flasks containing sodium citrate when used for antigen production. The processing of the citrate blood is started immediately after the blood is harvested, by centrifuging and removing the serum, then washing the cells six times in physiological saline. One volume of the washed red cells is then added to approximately 30 volumes of carbon dioxide saturated, ice-cold, distilled water, which is then agitated, and placed in a refrigerator overnight. A pink-white precipitate forms and settles to the bottom of the container. The following day the supernatant fluid is drawn off. The precipitate is centrifuged and washed in ice-cold, distilled water until the supernatant fluid contains no color. This usually takes three or more washings. The carbon dioxide precipitate is soluble in salt water and usually shows an acid reaction of about pH 4. The amount of washed, packed precipitate is measured, the acidity neutralized with 1.2 percent sodium bicarbonate, and physiological saline added in sufficient quantity to make a standard concentration of antigen equal to three times the packed precipitate volume. This finished product is then pipetted into ampules, lyophilized, vacuum sealed and stored at -50° C. to -70° C. until used.

The antigen to be used is restored to its original volume with distilled water, thoroughly agitated, then centrifuged slowly to settle any undissolved particles, and the supernatant material is drawn off for complement-fixation work.

Satisfactory antigens have been prepared according to the above-described methods and stored for 2 years at -50° C. to -70° C. without any appreciable loss of antigenicity; however, duplicate samples stored at refrigerator temperatures appeared to show a marked loss of antigenicity after a few months in storage.

References

1. Mohler, J. R. Report of the Chief of the U. S. Bureau of Animal Industry. p. 43, 1931-1932.

2. Rees, Charles W. and Mohler, William M., Journal A.V.M.A., Vol. 38, No. 5, pp. 669, Nov. 1934.

3. Kent, J. F. and Rein, C. R., The complement-fixation test for the serum diagnosis of malaria. Personal Communication.

4. Heidelberger, Michael and Mayer, Manfred M., Normal human stroma as antigens for complement fixation in the sera of patients with relapsing vivax. Science Magazine, Vol. 100, No. 2599, pp. 359-360, Oct. 20, 1944.

5. Lotze, Joh, Variables and constants in experimental bovine anaplasmosis and their relationship to chemotherapy. Am. Jnl. Vet. Research, Vol. 8, No. 28, pp. 263-274, July 1947.

Chart 1

ANAPLASMOSIS VACCINATION EXPOSURES AND RESULTS FROM VACCINATION TEST NO. 1

	VACCINATION				EXPOSURE			POST-EXPOSURE DATA					
					10-10-45 from 2596			Incu-	Maximum	Red cell volume		Temperature	
		Pas-			Method:		Para-	bation	parasite	Before	Minimum		Days
Cow	Antigen	sage	Type of		Dose	R.C.V.	site	period	count	exposure	post-ex-		after
number	source	number	antigen	Date	Dosage cc	percent	count	Days	Percent	Percent	posure %	Maximum	exposure
				7-18-45									
			Crude	7-25-45		I.V.							
2224	2616	10th	blood	8-2-45	356cc	400	9.5	20	9	3.5	41.0	19.0	102.4	
				8-17-45									
				8-24-45									
				8-30-45									
			CO2	9-6-45		IV.							
2130	2160	13th	spleen	9-13-45	2775cc	400	9.5	20	14	8.0	38.5	14.5	102.8	
			Crude			I.V.								
2132	2953	7th	blood	7-9-45	200cc	400	9.5	20	11	12.0	55.0	24.0	102.0	
				8-17-45									
			Crude	8-24-45		I.V.							
2222	2160	13th	blood	8-30-45	739cc	400	9.5	20	11	22.0	43.0	16.0	105.2	24th
			Crude			I.V.								
2114	2892	8th	blood	7-4-45	200cc	400	9.5	20	9	21.0	44.0	14.5	104.6	14th
	2160	13th		8-30-45									
	2526	13th	CO2	9-6-45		I.V.							
2206	2536	14th	blood	9-13-45	684cc	400	9.5	20	12	20.0	43.5	10.5	105.0	19th
						I.V.								
2375	CONTROL					400	9.5	20	3	21.0	44.0	10.0	105.4	8th

Exposure Results of Anaplasmosis Vaccine Test Number Two Animals Grouped According to Degree of Infection

Group No.	Cow No.	Incubation period	Parasite count	Red Cell Volume: Normal	Red Cell Volume: Minimum	Red Cell Volume: Cell Loss Percent	Temperature: Maxum.	Temperature: Days with Temp.	Days off Feed	Sub-inoculation Results	Mated Pairs of Cows	Vaccine Type
	2634	0	0	43.7	39.0	10.8	--	0	0	Negative	2	Blood Vaccine
Group 1	2152	0	0	42.7	39.5	7.5	--	0	0	Negative	4	Re-vacc.spleen vac.
Negative	2627	0	0	43.4	38.5	11.3	--	0	0	Negative	4	Blood vaccine
Cases	2521	0	0	38.7	32.0	17.4	--	0	0	Negative	6	Spleen crude vac.
Average		0	0	42.1	37.2	11.8	--	0	0			
Group 2	2544	32.	1.2	47.7	38.2	20.0	--	0	0	Positive	1	Blood vaccine
Non-clin-	2169	30.	0.6	42.8	24.8	42.1	--	0	0	Positive	3	Blood vaccine
ical case	2164	26.	3.2	46.0	23.5	49.0		0	0	Positive	5	Blood vaccine
Average		29.3	1.7	44.1	28.8	37.0	--	0	0			
Group 3												
showed temp	2533	30	22.2	43.7	19.2	56.1	104.0	2	0	None	1	)Re-vaccinated
not off feed	2654	32	10.3	41.0	15.0	63.5	106.0	3	0	None	2	)spleen vaccines
Average		31	16.2	42.3	17.1	59.8	105.0	2.5	0			
Group 4	2165	26	20.	41.7	13.5	67.7	105.0	6	3	None	3	)Re-vaccinated
showed temp	2107	32	15.	36.7	9.5	74.2	106.8	2	8	None	5	)spleen vaccines
off feed	2510	30	17.2	37.8	11.5	69.6	104.8	6	6	None	6	Spleen CO2 vaccine
Average		29.3	17.4	38.7	11.5	70.7	105.5	4.7	5.7			
Group 5	2264	32	16.6	40.0	9.5	76.3	106.0	6	6	None		
Controls	2551	32	16.3	38.8	14.5	62.7	105.8	3	2	None		
	2697	30	12.9	55.9	18.0	67.8	106.0	3	7	None		
Average		31.3	15.3	44.9	14.0	68.9	105.9	4	5			

Table Showing Relationship of Infecting Dose to the Incubation Period

Source Carrier Animal	Subinoculated into Animal Number	Dose	Incubation Period
2026	2940	10 cc	33 days
"	2956	10 cc	39 "
"	2943	10 cc	33 "
"	2793	50 cc	39 "
1368	2976	280 cc	24 "
2580	3055	500 cc	14 "
"	3017	1000 cc	11 "
"	3027	1000 cc	9 "
2026	3023	1800 cc	12 "
3055	3022	2800 cc	8 "
Acute Animals			
2942	2941	500 cc	1 day
2868	2856	2000 cc	1 "

Table Showing Approximate Pattern of Parasite Increase in the Red Blood Cells

Percent Parasitized Cells	Average Number of Bodies Per Infected Cell	Infected Cells Showing Approximate Bodies Per Cell - Percentage							
		1 Body Per Cell:	2 Bodies: Per Cell:	3 Bodies: Per Cell:	4 Bodies: Per Cell:	5 Bodies: Per Cell:	6 Bodies: Per Cell:	7 Bodies: Per Cell:	
5	1.	100							
10	1.12	88.	12.						
20	1.26	77	20	3					
30	1.34	70	24	4.0	1.0				
40	1.43	66	26	6.4	1.0	0.5			
50	1.60	54.0	28.	10.	3.5	1.1	0.1		
60	1.83	40.0	30.	18	4.	2.	0.5		
70	2.06	38.	38	16	7	2.	1.0		
80	2.56	24	25	30	13	6	1.	0.6	

7120 normal cells counted

2827 infected cells counted